Amendments to the Claims

The listing of claims will replace all prior versions, and listings of claims in the application.

1. (Currently Amended) A multiple inducible gene regulation system comprising a plurality of individually operable gene regulation systems, wherein:

a) each individually operable gene regulation system comprises:

i) one or more polynucleotides encoding a receptor complex comprising:

A) a DNA binding domain;

B) a Group H nuclear receptor ligand binding domain and a nuclear receptor ligand binding domain capable of forming a dimer with the Group H nuclear receptor ligand binding domain; and

C) a transactivation domain;

ii) a ligand;

iii) a polynucleotide comprising:

A) an exogenous or endogenous polynucleotide; and

B) a response element;

wherein:

A) the exogenous or endogenous polynucleotide is operatively linked to the response element; and

B) binding of the DNA binding domain to the response element in the presence or absence of the ligand results in activation or suppression of the exogenous or endogenous polynucleotide; and

~~C) the ligand binding domain comprises a ligand binding domain from a nuclear steroid receptor; and~~

b) each individually operable gene regulation system is orthogonal to the other individually operable gene modulation system present in the multiple inducible gene modulation system.

2. (Currently Amended) The multiple inducible gene regulation system of claim 1, wherein each operable gene regulation system comprises

a) i) a first gene expression cassette comprising a polynucleotide that encodes a polypeptide comprising a transactivation domain, a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated; and a Group H nuclear ~~steroid~~ receptor ligand binding domain,

ii) a ligand, and

iii) a second gene expression cassette comprising: A) a response element recognized by the DNA-binding domain of the encoded polypeptide of the first gene expression cassette; B) a promoter that is activated by the transactivation domain of the encoded polypeptide of the first gene expression cassette; and C) a gene whose expression is to be modulated;

b) i) a first gene expression cassette comprising a polynucleotide that encodes a polypeptide comprising a transactivation domain, a DNA-binding domain that

recognizes a response element associated with a gene whose expression is to be modulated; and a Group H nuclear ~~steroid~~ receptor ligand binding domain,

ii) a ~~second~~ nuclear ~~steroid~~ receptor ligand binding domain selected from the group consisting of a vertebrate retinoid X receptor ligand binding domain, an invertebrate retinoid X receptor ligand binding domain, an ultraspiracle protein ligand binding domain, and a chimeric ligand binding domain comprising two polypeptide fragments, wherein the first polypeptide fragment is from a vertebrate retinoid X receptor ligand binding domain, an invertebrate retinoid X receptor ligand binding domain, or an ultraspiracle protein ligand binding domain, and the second polypeptide fragment is from a different vertebrate retinoid X receptor ligand binding domain, invertebrate retinoid X receptor ligand binding domain, or ultraspiracle protein ligand binding domain,

iii) a ligand, and

iv) a second gene expression cassette comprising: A) a response element recognized by the DNA-binding domain of the encoded polypeptide of the first gene expression cassette; B) a promoter that is activated by the transactivation domain of the encoded polypeptide of the first gene expression cassette; and C) a gene whose expression is to be modulated; or

c) i) a first gene expression cassette comprising a polynucleotide that encodes a first polypeptide comprising a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated and a Group H nuclear ~~steroid~~ receptor ligand binding domain,

ii) a second gene expression cassette comprising a polynucleotide that encodes a second polypeptide comprising a transactivation domain and a Group H nuclear ~~steroid~~ receptor ligand binding domain,

iii) a ligand, and

iv) a third gene expression cassette comprising: A) a response element recognized by the DNA-binding domain of the first polypeptide of the first gene expression cassette; B) a promoter that is activated by the transactivation domain of the second polypeptide of the second gene expression cassette; and C) a gene whose expression is to be modulated ~~,~~

~~wherein one of the nuclear steroid receptor ligand binding domains of c)i) or c)ii) is a Group H nuclear steroid receptor ligand binding domain~~.

3. (Original) A virus comprising the multiple gene regulation system of claim 1.

4. (Original) A cell comprising the multiple gene regulation system of claim 1.

5. (Cancelled)

6. (Canceled)

7. (Original) The multiple inducible gene regulation system of claim 1, wherein one or more of the polynucleotides encoding a receptor complex encodes a non-mammalian receptor complex.

8. (Currently Amended) The multiple inducible gene regulation system of claim 1 [[6]], wherein the receptor complex is an ecdysone receptor complex.

9. (Currently Amended) A multiple inducible gene regulation system which comprises a plurality of individually operable gene regulation systems wherein:

a) each individually operable gene regulation system comprises:

i) one or more receptor complexes, each comprising:

A) a DNA binding domain;

B) a Group H nuclear receptor ligand binding domain and a nuclear receptor ligand binding domain capable of forming a dimer with the Group H nuclear receptor ligand binding domain; and

C) a transactivation domain;

ii) a ligand;

iii) a polynucleotide comprising:

A) an exogenous or endogenous gene; and

B) a response element;

wherein:

A) the exogenous or endogenous gene is under the control of the response element; and

B) binding of the DNA binding domain to the response element in the presence or the absence of the ligand results in activation or suppression of the gene; and

~~C) the ligand-binding domain comprises a ligand binding domain from a nuclear steroid receptor; and~~

b) each individually operable gene regulation system is orthogonal to the other individually operable gene regulation systems present in the multiple inducible gene regulation system.

10. (Currently Amended) The multiple inducible gene regulation system of claim 9, wherein each operable gene regulation system comprises

a) i) a polypeptide comprising a transactivation domain, a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated; and a Group H nuclear ~~steroid~~ receptor ligand binding domain,

ii) a ligand, and

iii) a gene expression cassette comprising: A) a response element recognized by the DNA-binding domain of the polypeptide of a)i); B) a promoter that is activated by the transactivation domain of the polypeptide of a)i); and C) a gene whose expression is to be modulated;

b) i) a polypeptide comprising a transactivation domain, a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated; and a Group H nuclear ~~steroid~~ receptor ligand binding domain,

ii) a ~~second~~ nuclear receptor ligand binding domain selected from the group consisting of a vertebrate retinoid X receptor ligand binding domain, an invertebrate retinoid X receptor ligand binding domain, an ultraspiracle protein ligand binding domain, and a chimeric ligand binding domain comprising two polypeptide fragments, wherein the first polypeptide fragment is from a vertebrate retinoid X receptor ligand binding domain, an invertebrate retinoid X receptor ligand binding domain, or an

ultraspiracle protein ligand binding domain, and the second polypeptide fragment is from a different vertebrate retinoid X receptor ligand binding domain, invertebrate retinoid X receptor ligand binding domain, or ultraspiracle protein ligand binding domain,

iii) a ligand, and

iv) a gene expression cassette comprising: A) a response element recognized by the DNA-binding domain of the polypeptide of b)i); B) a promoter that is activated by the transactivation domain of the polypeptide of b)i); and C) a gene whose expression is to be modulated; or

c) i) a first polypeptide comprising a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated and a Group H nuclear ~~steroid~~ receptor ligand binding domain,

ii) a second polypeptide comprising a transactivation domain and a nuclear steroid receptor ligand binding domain,

iii) a ligand, and

iv) a gene expression cassette comprising: A) a response element recognized by the DNA-binding domain of the first polypeptide of c)i); B) a promoter that is activated by the transactivation domain of the second polypeptide of c)ii); and C) a gene whose expression is to be modulated~~,~~

~~wherein one of the nuclear steroid receptor ligand binding domains of c)i) or c)ii) is a Group H nuclear steroid receptor ligand binding domain~~.

11. (Original) A virus comprising the multiple gene regulation system of claim 9.

12. (Original) A cell comprising the multiple gene regulation system of claim 9.

13. (Cancelled)

14. (Canceled)

15. (Currently Amended) The multiple inducible gene regulation system of claim [[14]] 9, wherein the Group H receptor complex is an ecdysone receptor complex.

16.-20. (Cancelled)

21. (Withdrawn) A multiple inducible gene regulation system, the system comprising:

a) a first, second, and third hybrid peptide, wherein:

i) the first hybrid peptide comprises a first DNA binding domain and a first ligand binding domain;

ii) the second hybrid peptide comprises a second DNA binding domain and a second ligand binding domain;

iii) the third hybrid peptide comprises a transactivation domain and a third ligand binding domain;

b) at least a first and second ligand; and

c) a first and second polynucleotide of interest operably linked, respectively, to a first and second promoter construct;

wherein:

I. the first and third hybrid peptides dimerize to form a first dimer in the presence of the first ligand,

II. the second and third hybrid peptides dimerize to form a second dimer in the presence of the second ligand; and

III. the first and second dimer bind to the first and second promoter construct, respectively, resulting in transcription of the first and second polynucleotides of interest within the same cell.

22. (Withdrawn) The system of claim 21, wherein the transcription of said first and second polynucleotides of interest is orthogonal to each other.

23. (Withdrawn) The system of claim 22 wherein the transcription of said first and second polynucleotides of interest is fully orthogonal to each other.

24. (Withdrawn) The system of claim 21, wherein at least one of the first and second polynucleotides of interest is exogenous to the cell.

25. (Withdrawn) The system of claim 24, wherein the first and second polynucleotides of interest are exogenous to the cell.

26. (Withdrawn) The system of claim 21, wherein the first and second DNA binding domains are different from one another and each comprises one of a Ga14 DNA binding

domain, a LexA DNA binding domain, a transcription factor DNA binding domain, a Group H nuclear receptor DNA binding domain, a steroid/thyroid hormone nuclear receptor superfamily DNA binding domain, and a bacterial LacZ DNA binding domain.

27. (Withdrawn) The system of claim 26, wherein the first and second DNA binding domains comprise one of a Ga14 DNA binding domain and a LexA DNA binding domain, wherein if the first DNA binding domain is a Ga14 DNA binding domain, the second DNA binding domain is a LexA DNA binding domain, and wherein if the first DNA binding domain is a LexA DNA binding domain, the second DNA binding domain is a Ga14 DNA binding domain.

28. (Withdrawn) The system of claim 21, wherein the transactivation domain comprises a Group H nuclear receptor transactivation domain, a viral protein transactivation domain, a steroid/thyroid hormone nuclear receptor transactivation domain, a synthetic transactivation domain, a chimeric transactivation domain, a polyglutamine transactivation domain, a basic or acidic transactivation domain, a VP 16 transactivation domain, a GAL4 transactivation domain, an NF-kB transactivation domain, a BP64 transactivation domain, a B42 transactivation domain, or a p65 transactivation domain.

29. (Withdrawn) The system of claim 28, wherein the transactivation domain is a VP16 transactivation domain.

30. (Withdrawn) The system of claim 21, wherein the first and second ligand binding domains comprise a Group H nuclear receptor ligand binding domain, and the third ligand binding domain is a vertebrate retinoid X receptor ligand binding domain, an invertebrate retinoid X receptor ligand binding domain, an ultraspiracle protein ligand binding domain, or a chimeric ligand binding domain comprising two polypeptide fragments, wherein the first polypeptide fragment is from a vertebrate retinoid X receptor ligand binding domain, an invertebrate retinoid X receptor ligand binding domain, or an ultraspiracle protein ligand binding domain, and the second polypeptide fragment is from a different vertebrate retinoid X receptor ligand binding domain, invertebrate retinoid X receptor ligand binding domain, or ultraspiracle protein ligand binding domain.

31. (Withdrawn) The system of claim 30, wherein the first and second ligand binding domains comprise ecdysone receptor ligand binding domains from different invertebrate species.

32. (Withdrawn) The system of claim 30, wherein the third ligand binding domain comprises a vertebrate retinoid X receptor ligand binding domain.

33. (Withdrawn) The system of claim 30, wherein the third ligand binding domain is a chimeric ligand binding domain comprising a polypeptide from a vertebrate retinoid X receptor ligand binding domain and a polypeptide from an ultraspiracle ligand binding domain.

34. (Withdrawn) The system of claim 30, wherein at least one of the first and second ligand binding domains is a Dipteran ecdysone receptor ligand binding domain.

35. (Withdrawn) The system of claim 30, wherein at least one of the first and second ligand binding domains is a Lepidopteran ecdysone receptor ligand binding domain.

36. (Withdrawn) The system of claim 30, wherein the first and second ligand binding domains comprise one of a Dipteran ecdysone receptor ligand binding domain and a Lepidopteran ecdysone receptor ligand binding domain, wherein if the first ligand binding domain is a Dipteran ecdysone receptor ligand binding domain, the second ligand binding domain is a Lepidopteran ecdysone receptor ligand binding domain, and wherein if the first ligand binding domain is a Lepidopteran ecdysone receptor ligand binding domain, the second ecdysone receptor ligand binding domain is a Dipteran ecdysone receptor ligand binding domain.

37. (Withdrawn) The system of claim 36, wherein the first and second ligand binding domains are encoded, respectively, by the nucleotide sequence shown in SEQ ID NO: 1 and the nucleotide sequence shown in SEQ ID NO:4, or the nucleotide sequence shown in SEQ ID NO:4 and the nucleotide sequence shown in SEQ ID NO:1.

38. (Withdrawn) The system of claim 21, wherein the first and second ligand binding domains are encoded, respectively, by the nucleotide sequence shown in SEQ ID NO: 1 and the nucleotide sequence shown in SEQ ID NO:4, or the nucleotide sequence shown

in SEQ ID NO:4 and the nucleotide sequence shown in SEQ ID NO:l, and the third ligand binding domain is encoded by the nucleotide sequence shown in SEQ ID NO:6.

39. (Withdrawn) The system of claim 30, wherein at least one of the first and second ligand binding domains is a Homopteran ecdysone receptor ligand binding domain.

40. (Withdrawn) The system of claim 30, wherein the first and second ligand binding domains comprise one of a Lepidopteran ecdysone receptor ligand binding domain and a Homopteran ecdysone receptor ligand binding domain, wherein if the first ligand binding domain is a Lepidopteran ecdysone receptor ligand binding domain, the second ligand binding domain is a Homopteran ecdysone receptor ligand binding domain, and if the first ligand binding domain is a Homopteran ecdysone receptor ligand binding domain, the second ligand binding domain is a Lepidopteran ecdysone receptor ligand binding domain.

41. (Withdrawn) The system of claim 40, wherein the first and second ligand binding domains are encoded, respectively, by the nucleotide sequence shown in SEQ ID NO: 14 and by the nucleotide sequence shown in SEQ ID NO: 15, or by the nucleotide sequence shown in SEQ ID NO: 15 and by the nucleotide sequence shown in SEQ ID NO:14.

42. (Withdrawn) The system of claim 21, wherein the first and second ligand binding domains are encoded, respectively, by the nucleotide sequence shown in SEQ ID NO: 14 and by the nucleotide sequence shown in SEQ ID NO: 15, or by the nucleotide sequence

shown in SEQ ID NO: 15 and by the nucleotide sequence shown in SEQ ID NO:14, and wherein the third ligand binding domain is encoded by the nucleotide sequence shown in SEQ ID NO: 16.

43. (Withdrawn) The system of claim 21, wherein the first and second ligand binding domains are each encoded by one of the nucleotide sequences shown in SEQ ID NO: 1, SEQ ID NO:4, SEQ ID NO: 14, or SEQ ID NO:15, and the third ligand binding domain is encoded by one of the nucleotide sequence shown in SEQ ID NO:6 or SEQ ID NO:16.

44. (Withdrawn) A virus comprising the system of claim 21.

45. (Withdrawn) A cell comprising the system of claim 21.

46. (Withdrawn) A dual inducible gene regulation system, the system comprising:

a) a first, second, and third hybrid peptide, wherein:

   i) the first hybrid peptide comprises a first DNA binding domain and a first ligand binding domain;

   ii) the second hybrid peptide comprises a second DNA binding domain and a second ligand binding domain;

   iii) the third hybrid peptide comprises a transactivation domain and a third ligand binding domain;

b) a first and second ligand; and

c) a first and second polynucleotide of interest operably linked to a first and second promoter construct;

wherein:

I. the first and third hybrid peptides dimerize to form a first dimer in the presence of the first ligand;

II. the second and third hybrid peptides dimerize to form a second dimer in the presence of the second ligand; and

III. the first and second dimer bind to the first and second promoter construct, respectively, resulting in transcription of the first and second polynucleotides of interest within the same cell.

47. (Currently Amended) A dual inducible gene regulation system comprising two individually operable gene regulation systems wherein:

a) each individually operable gene regulation system comprises:

i) one or more polynucleotides encoding a receptor complex comprising:

A) a DNA binding domain;

B) a Group H nuclear receptor ligand binding domain and a nuclear receptor ligand binding domain capable of forming a dimer with the Group H nuclear receptor ligand binding domain; and

C) a transactivation domain;

ii) a ligand;

iii) a polynucleotide comprising:

A) an exogenous or endogenous polynucleotide; and

B) a response element;

wherein:

A) the exogenous or endogenous polynucleotide is operatively linked to the response element; and

B) binding of the DNA binding domain to the response element in the presence or absence of the ligand results in activation or suppression of the exogenous or endogenous polynucleotide; and

~~C) the ligand binding domain comprises a ligand binding domain from a nuclear steroid receptor; and~~

b) each individually operable gene regulation system is orthogonal to the other individually operable gene modulation system present in the dual inducible gene modulation system.